(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 0 795 562 A1

(12) **EUROPEAN PATENT APPLICATION**
published in accordance with Art. 158(3) EPC

(43) Date of publication:
**17.09.1997 Bulletin 1997/38**

(21) Application number: **95936110.6**

(22) Date of filing: **07.11.1995**

(51) Int. Cl.[6]: **C07K 14/575**, A61K 38/22

(86) International application number:
**PCT/JP95/02269**

(87) International publication number:
**WO 96/14336 (17.05.1996 Gazette 1996/22)**

(84) Designated Contracting States:
**AT BE CH DE DK ES FR GB GR IE IT LI LU MC NL PT SE**

(30) Priority: **07.11.1994 JP 272069/94**

(71) Applicant: **KYOWA HAKKO KOGYO CO., LTD.**
**Chiyoda-ku, Tokyo 100 (JP)**

(72) Inventors:
- **YANO, Keiichi**
  **Kanagawa 215 (JP)**
- **YAMASAKI, Motoo**
  **Tokyo 194 (JP)**
- **MORIYAMA, Takahiro**
  **Shizuoka 411 (JP)**
- **ANDO, Masaaki**
  **Hiroshima 739-21 (JP)**

(74) Representative: **Casalonga, Axel et al**
**BUREAU D.A. CASALONGA - JOSSE**
**Morassistrasse 8**
**80469 München (DE)**

# (54) NOVEL OXYNTOMODULIN

(57) A peptide represented by the following formula (I) or a pharmacologically acceptable salt thereof:
H-His-Ser-Gln-Gly-Thr-Phe-Thr-Asn-Asp-Tyr-Ser-Lys-Tyr-Leu-Glu-Thr-Arg-Arg-Ala-Gln-Asp-Phe-Val-Gln-Trp-Leu-Met-Asn-Ser-Lys-Arg-Ser-Gly-Gly-Pro-Thr-OH. It has cardiotonic and insulin release accelerator effects and is useful for preventing or treating heart diseases or diabetes.

EP 0 795 562 A1

## Description

### TECHNICAL FIELD

The present invention relates to a novel peptide or pharmaceutically acceptable salts thereof, which have cardiotonic activity and insulin release-promoting activity and are useful for prevention or treatment of cardiopathy or diabetes.

### BACKGROUND ART

Oxyntomodulin (hereinafter referred to as OXM) is a peptide which is produced post-translational processing of proglucagon, like glucagon (peptide composed of 29 amino acids), glucagon-like peptides and glicentin, and is recognized to exist in intestines and hypothalamus. OXM has a structure of glucagon or glucagon-like peptide to which is added 7 or 8 amino acid residues at its carboxyl terminal, and its structures in porcine (D. Bataille et al., FEBS Lett., 146, 73-78, 1982), in bovine (J.M. Conlon et al., Regul. Pept., 11, 309-320, 1985), in rat (A. Kervran et al., Endocrinol., 121, 704-713, 1987), in shark (J.M. Conlon et al., Biochem. J., 245, 851-855, 1987), in canine (Y. Shinomura et al., Regul. Pept., 23, 299-308, 1988), in bullfrog (H.G. Pollock et al., J. Biol. Chem., 263, 9746-9751, 1988), and in alligator (H.G. Pollock et al., Gen. Comp. Endocrinol., 69, 133-140, 1988) have been clarified at present. The primary structures of OXM of every species are similar to each other, having neither cysteine residue nor proline residue in the molecule. Regarding the physiological activities of OXM, for example, its somatostatin secretion-promoting activity (T. Tani et al., Biochim. Biophys. Acta, 1095, 249-254, 1991), its gastric acid secretion-inhibiting activity (T.M. Biedzinski et al., Peptides, 8, 967-972, 1987), and its insulin secretion-promoting activity (C. Jarrousse et al., Endocrinol., 115, 102-105, 1984) have heretofore been reported. It is believed that OXM may exhibit its physiological functions via a receptor which is different from that for glucagon (C. Depigny et al., C.R. Acad. Sci. III (France), 299, 677-680, 1984). However, This belief has not been completely clarified as yet. Though being similar to glucagon in its primary structure, OXM obviously differ from glucagon, for example, in its distribution in tissue and its gastric acid secretion-inhibiting activity. To further clarify the physiological activities of OXM, the analysis of its structures in further lower animals is also important. For example, regarding the effect of calcitonin on calcium concentration on the rat plasma, it is known that the effect of eel calcitonin is obviously higher than that of rat, porcine and human calcitonins (M. Otani et al., J. Biochem., 79, 345-352, 1976; M. Azria, Prog. Clin. Biochem. Med., 9, 1-33, 1989). However, the presence of eel OXM, the structure thereof and the physiological activities thereof are not known up to these days.

### DISCLOSURE OF THE INVENTION

We, the present inventors have variously studied substances existing in eel intestines, and as a result, have found a novel factor having atrial contractile activity. As a result of having further studied it, we have found that this factor is a novel peptide, while having a cardiotonic activity and an insulin release-promoting activity, and is therefore useful as a medicine for cardiopathy, diabetes, etc. On the basis of these findings, we have completed the present invention.

Specifically, the present invention relates to a peptide of the following formula (I):

H-His-Ser-Gln-Gly-Thr-Phe-Thr-Asn-Asp-Tyr-Ser-Lys-Tyr-Leu-Glu-Thr-Arg-Arg-Ala-Gln-Asp-Phe-Val-Gln-Trp-Leu-Met-Asn-Ser-Lys-Arg-Ser-Gly-Gly-Pro-Thr-OH (I)

or pharmaceutically acceptable salts thereof.

The abbreviations for the corresponding amino acids in formula (I) are generally used in this technical field, which are as follows:

| | |
|---|---|
| Ala: | L-alanine |
| Arg: | L-arginine |
| Asn: | L-asparagine |
| Asp: | L-aspartic acid |
| Gln: | L-glutamine |
| Glu: | L-glutamic acid |
| Gly: | Glycine |
| His: | L-histidine |
| Leu: | L-leucine |
| Lys: | L-lysine |
| Met: | L-methionine |
| Phe: | L-phenylalanine |
| Pro: | L-proline |
| Ser: | L-serine |
| Thr: | L-threonine |

Trp: L-tryptophan
Tyr: L-tyrosine
Val: L-valine

The pharmaceutically acceptable salts of the peptide of formula (I) include salts with inorganic acids such as hydrochlorides, sulfates and phosphates; and salts with organic acid such as acetates, maleates, fumarates, tartrates, citrates, lactates and methanesulfonates.

The peptide of the present invention is a novel OXM, the structure of which is different from those of any other known OXMs. Specifically, in known OXMs, the 15th amino acid residue from the amino terminal is Asp and the 35th residue therefrom is Asn or Ile, while in the peptide of the present invention is characterized in that the 15th residue thereof is Glu and the 35th residue thereof is Pro.

The peptide of the present invention can be obtained according to a method of collecting it from cells that produce the said peptide, or according to organic synthetic means. The cells that produce the said peptide may include the eel intestinal tissue cells, the cells as grown by cultivating them, the cells as transformed according to genetic recombination to produce the intended peptide, etc.

To extract and isolate the peptide of the present invention from eel intestines, eel intestines are homogenized in suitable acidic solution, for example, in an aqueous solution of acetic acid, hydrochloric acid or the like, insoluble substances are removed from the resulting homogenate through centrifugation to obtain a liquid extract, and thereafter this liquid extract is subjected to a combination of any treatments generally employed for isolation and purification of peptides such as fractional precipitation with an organic solvent, solvent extraction, dialysis, ultrafiltration, ion-exchange chromatography, adsorption chromatography, gel permeation chromatography, high-performance liquid chromatography, crystallization and electrophoresis. Thereby obtaining the substance having the corresponding molecular weight.

To obtain the peptide of the present invention according to genetic recombination, DNA parts each coding for the N-terminal side and the C-terminal side of the said peptide are separately synthesized, then cloned separately, linked together optionally via an oligonucleotide linker, and introduced into a suitable expression vector according to an ordinary means, and then a host such as *Escherichia coli*, yeast and animal cells is transformed with the obtained vector, and the resulting transformant is incubated. As the means of expressing genes, there have heretofore been developed direct methods, secretion methods and fusion methods. Any of these methods may be employed in the present invention. For example, referred to are the methods described in J. Sambrook et al., "Molecular Cloning 2nd Edition", Cold Spring Harbor Lab. Press, New York (1989). To isolate and purify the peptide of the present invention from these cells, employable is any combination of the above-mentioned ordinary means for the isolation and purification of peptides.

It is also possible to chemically synthesize the peptide of the present invention from amino acids on the basis of the amino acid information of the said peptide. For such synthesis, employable are any ordinary peptide synthesizing methods, such as liquid phase methods and solid phase methods. For example, referred to are the methods described in H. Yajima & S. Yanagihara, Seikagaku Jikken Kouza (I): Tanpakushitu no Kagaku, Vol. 4 (edited by the Japanese Biochemical Society, published by Tokyo Kagaku Dojin, 1977); and N. Izumiya et al., "Pepuchido Gousei no Kiso to Jikken" (published by Maruzen, 1985).

Where solid phase methods are employed for obtaining the peptide, p-benzyloxybenzyl alcohol-type resins (e.g., polystyrene) are preferred as the support. For these, for example, commercial products comprising a resin of that type, to which is bonded an $\alpha$-amino-protected amino acid via ester bonding, are available. For these, preferably, the $\alpha$-amino groups of the amino acid to be used are protected with 9-fluorenylmethoxycarbonyl (Fmoc) groups; the guanidino groups of arginine is with 2,2,5,7-pentamethylchroman-6-sulfonyl (Pmc) groups; the $\beta$- and $\gamma$-carboxylic acids of aspartic acid and glutamic acid, and the hydroxyl groups of serine, threonine and tyrosine are with t-butyl (tBu) groups; the $\beta$- and $\gamma$-amido groups of asparagine and glutamine, and the imidazole groups of histidine are with trityl (Trt) groups; and the $\varepsilon$-amino groups of lysine is with t-butyloxycarbonyl (Boc) groups. The condensation of the protected amino acids is preferably conducted, using a condensing agent, such as dicyclohexylcarbodiimide (DCC) and benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) in the presence of N-hydroxybenzotriazole (HOBt). As the solvent preferably used is N,N-dimethylformamide (DMF), and the deprotection of Fmoc groups is preferably effected with N-methylmorpholine (NMM). The amino acids are prolonged in order to give a protected peptide resin, which is processed with an acid such as trifluoroacetic acid (TFA) in the presence of a sulfur-containing compound such as thioanisole, ethanediol, ethylmethylsulfide and thiophenol, to give a crude synthetic peptide. In ordinary condensation, the protected amino acid, the condensing agent and HOBt are used each in an amount of from 1 to 15 equivalents relative to the hydroxyl groups of the resin or to the amino acids as bonded first to the resin. Such ordinary condensation is effected at room temperature for from 30 minutes to 5 hours. To release the synthetic peptide from the resin, the resin may be processed at room temperature for from 1 to 10 hours. To purify the thus-obtained peptide, employable is any combination of the above-mentioned ordinary means for isolation and purification of peptides.

Now, the pharmaceutical effects of the peptide (eel OXT) of formula (I) are mentioned to the following Test Examples.

Test Example 1 Effect of OXT in Eel Atrium

An atrium was taken out of an eel, and one end of said atrium was fixed to a the bottom of syringe filled with 1.5 ml of Ringer's solution in which was introduced a mixed gas of oxygen and carbon dioxide (95:5), while the other end thereof was connected to a tension transducer (NEC) via a silk thread. 15 µl of an aqueous solution of OXM as diluted with distilled water to have a varying concentration was added to said Ringer's solution, whereupon the tension to be generated by the atrial contraction was measured through the tension transducer. OXM used herein was the product as produced in Example 2. During this measurement, the atrial rate was also counted. Fig. 1 shows the relationship between the concentration of OXM added and the atrial tension measured, and Fig. 2 shows the relationship between the OXM concentration and the atrial rate counted.

These data verify that OXM dose-dependently increases the atrial systole and the atrial rate.

Test Example 2 Effect of OXM on Plasma Insulin Concentration of Anesthetized Rat

Male SD rats (370 to 450 g) were bred with no feed for from 15 to 20 hours, and then Na pentobarbital (50 mg/kg) was intra-abdominally administered thereto. A blood-collecting cannula was inserted into the carotid artery of each rat. A dosed cannula was inserted into the jugular vein of each rat. About 600 µl of blood was collected from each rat before the administration of a test chemical, and 5 minutes after each administration of a varying dose of the said test chemical. OXM obtained in Example 2 was added to physiological saline solution to adjust concentration of 1 mg/ml. The said OXM solution was diluted with a physiological saline solution, the thus-diluted solution was accumulatively administered to each rat. The dose of the solution was 1 mg/kg. The collected blood was centrifuged, and its insulin concentration was measured according to the Insulin-EIA Test (Wako Pure Chemicals). The data obtained are shown in Fig. 3.

The data verify that OXM increases the plasma insulin concentration at a dose of 0.01 mg/kg or more.

The peptide of formula (I) and pharmaceutically acceptable salts thereof can be administered through various administration routes for, such as injection, intra-mucosal administration and peroral administration, and can be used with various dosage forms according to the said administration routes. The said dosage forms are, for example, intravenous injection, intramuscular injection, subcutaneous injection and intracutaneous injection; preparations for such injection to be dissolved before use, such as suspensions and lyophilized powders; preparations for intra-mucosal application, such as suppositories and per-nasal preparations; or peroral preparations, such as tablets, capsules, granules and suspensions. To preparing these dose forms, employable are any known methods. If desired, these preparations may be formulated to any additives that are acceptable for medicines, such as preservatives, stabilizers, antioxidants, vehicles, binders, disintegrators, wetting agents, lubricants, colorants, aromatics, flavors, filming agents, suspending agents, emulsifiers, dissolution aids, buffers, isotonicators, plasticizers, surfactants and analgesics.

Example of the carriers which can be used are water, distilled water for injection, physiological saline solution, glucose, fructose, sucrose, mannitol, lactose, starch, cellulose, methyl cellulose, carboxymethyl cellulose, hydroxypropyl cellulose, glycerin, mannitol, xylitol, sorbitol, glucuronic acid, hyaluronic acid, heparin, chitin, chitosan, glycine, alanine, proline, serine, cysteine, aspartic acid, glutamic acid, lysine, arginine, human serum albumin, human serum globulin, collagen, gelatin, alginic acid, talc, sodium citrate, calcium carbonate, calcium hydrogenphosphate, magnesium stearate, urea, silicone resins, sorbitan fatty acid esters, glycerin fatty acid esters, ascorbic acid, α-tocopherol or the like.

The dose will vary, depending on the mode of administration, the age, the body weight and the symptoms of a patient, etc. However, it is generally appropriate to administer Compound(I) or a pharmaceutically acceptable salts thereof in a dose of 0.01 µg to 10 mg/60 kg/day.

## BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows the effect of Oxyntomodulin on eel atrial systole.

Fig. 2 shows the effect of Oxyntomodulin on eel atrial rate.

Fig. 3 shows the effect of Oxyntomodulin on rat plasma insulin concentration.

## BEST MODES OF CARRYING OUT THE INVENTION

Example 1 Isolation and Purification of Eel OXM

361 g of intestines as taken out of 150 eels were frozen with liquid nitrogen, ground, boiled in distilled water, homogenized in a mixture of 1 M acetic acid and 20 mM hydrochloric acid, and centrifuged to collect the resulting supernatant liquid. Acetone was added to a final concentration of 66 %, and again centrifuged to remove the precipitate. The resulting supernatant liquid was passed through Sep-Pak Plus C18 (Waters), whereupon the substance as held therein was eluted with 50 % acetonitrile, 10 % isopropyl alcohol and 0.1 % TFA (pH 2.2). The resulting eluate was purified through gel permeation chromatography using Toyopearl HW-40F (2.6 x 100 cm) to which was applied an eluent comprised of

1 M acetic acid and 10 % isopropyl alcohol at a flow rate of 1.5 ml/min. Among the resulting fractions, those that exhibited the eel atrial contractile activity as referred to in Test Example 1 were purified through 5-stage high-performance liquid chromatography mentioned below.

First Stage:

Column: Asahipak C8P-50 (4.6 x 250 mm, Asahi Chemical)
Eluent: Solution A (10 % isopropyl alcohol, 0.1 % TFA)
Solution B (50 % acetonitrile, 10 % isopropyl alcohol, 0.1 % TFA)
Mode: Linear gradient elution from solution A to solution B (for 50 minutes)

Second Stage:

Column: TSKgel ODS-80TM (4.6 x 250 mm, Toso)
Eluent: Solution A (15 % acetonitrile, 5 % isopropyl alcohol, 0.1 % TFA)
Solution B (35 % acetonitrile, 5 % isopropyl alcohol, 0.1 % TFA)
Mode: Linear gradient elution from solution A to solution B (for 100 minutes)

Third Stage:

Column: TSKgel CM-5PW (7.5 x 75 mm, Toso)
Eluent: Solution A (20 mM phosphate buffer, pH 6.8, 10 % isopropyl alcohol)
Solution B (20 mM phosphate buffer, pH 6.8, 10 % isopropyl alcohol, 350 mM sodium chloride)
Mode: Linear gradient elution from solution A to solution B (for 35 minutes)
Sodium chloride concentration at which OXM was eluted: 0.14 M

Fourth Stage:

Column: TSKgel ODS-80TM (4.6 x 150 mm, Toso)
Eluent: Solution A (24 % acetonitrile, 5 % isopropyl alcohol, 0.1 % TFA))
Solution B (34 % acetonitrile, 5 % isopropyl alcohol, 0.1 % TFA)
Mode: Linear gradient elution from solution A to solution B (for 50 minutes)
Retention Time of OXM: 31.5 minutes (organic solvent concentration: 33 %)

Fifth Stage:

Column: TSKgel ODS-80TM (4.6 x 150 mm, Toso)
Eluent: 27 % acetonitrile, 5 % isopropyl alcohol, 0.1 % TFA
Mode: Isocratic elution
Retention Time of OXM: 20.0 minutes

As a result of the purification mentioned above, obtained was about 7 µg of OXM that gave a single peak on its chromatographic pattern.

The physicochemical properties of OXM are mentioned below, which were measured using the apparatus mentioned below.

Mass spectrograph: Nippon Electronics' JMS-HX110A (measured by the FAB method)

For amino acid analysis of OXM, employed was the method of B.A. Bidlingmeyer et al. (J. Chromatogr., 336, 93, 1984). Hydrolysis was carried out in hydrochloric acid vapor at 110°C for 20 hours. The amino acid composition of the resulting hydrolysate was analyzed with Pico Tag amino acid analyzer (Waters).

Mass spectrometry: 4208 ± 1 (M + H) measured
Amino acid analysis: measured (theoretical)
Asx 3.5 (4), Glx 3.7 (4), Ser 4.3 (4), Gly 3.4 (3), His 1.1 (1), Arg 3.0 (3), Thr 4.5 (4), Ala 1.2 (1), Pro 1.2 (1)
Tyr 2.1 (2), Val 0.9 (1), Met 0.8 (1), Leu 2.0 (2), Phe 2.0 (2), Lys 1.8 (2)
(Trp is not analyzed; Asx is Asp or Asn; Glx is Glu or Gln.)

The primary structure of the amino acid sequence of OXM was determined through automatic Edman degradation

of OXM (using PPSQ-10 Protein Sequencer, produced by Shimadzu) as combined with FAB-MS spectrometry, and through automatic Edman degradation of partial peptides (these were obtained as a result of digestion of OXM with lysylendopeptidase in 0.1 M Tris-HCl buffer (pH 9) at 37°C for 6 hours followed by reversed-phase HPLC) as combined with FAB-MS spectrometry.

Example 2 Synthesis of OXM

Herein used was a Shimadzu's peptide synthesizer, PSSM8 along with Shimadzu's reagents and solvents. According to the Shimadzu's program for peptide synthesis, the synthesizer was run to synthesize the intended peptide. The condensation of the amino acids was effected under ordinary conditions according to the Fmoc method (N. Izumiya et al., Pepuchido Gousei no Kiso to Jikken, published by Maruzen, 1985).

Precisely, 50 mg of a carrier resin (of p-benzyloxybenzyl alcohol type) having 23 μmols of Fmoc-Thr-OH bonded thereto was put into the reactor of the automatic synthesizer, and processed as follows in accordance with the Shimadzu's synthesis program.

(a) The carrier resin was washed with DMF for 3 minutes, and the DMF wash was discharged.
(b) DMF with 30 % piperidine was added thereto, the resulting mixture was stirred for 4 minutes, and the DMF wash was discharged. This was repeated once again.
(c) The carrier resin was washed with DMF for 1 minutes, and the DMF wash was discharged. This was repeated five times. Thus was obtained a Thr-bonded carrier resin from which Fmoc was removed.
(d) 644 μl of DMF containing 184 μmols (62.0 mg) of Fmoc-Pro-OH, 184 μmols of PyBOP, 184 μmols of HOBt and 276 μmols of NMM was stirred for 3 minutes, and then added to the resin, and the resulting mixture was further stirred for 30 minutes. After this, the wash was discharged.
(e) The carrier resin was washed with DMF for 1 minute. This was repeated five times in all. Thus was formed Fmoc-Pro-Thr on the carrier. This was subjected to the above-mentioned steps (a) to (c) for washing and deprotection. Next, this was subjected to the step (d) for condensation where Fmoc-Gly-OH was used. Next, this was subjected to the washing step (e) to synthesize Fmoc-Gly-Pro-Thr on the carrier resin. Afterwards, the steps (a) to (e) were repeated in that order to finally obtain a carrier resin having a protected peptide bonded thereto. In the step (d), used were Fmoc-Gly-OH, Fmoc-Ser(tBu)-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Met-OH, Fmoc-Leu-OH, Fmoc-Trp-OH, Fmoc-Gln(Trt)-OH, Fmoc-Val-OH, Fmoc-Phe-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Gln(Trt)-OH, Fmoc-Ala-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Arg(Pmc)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Glu(OtBu)-OH, Fmoc-Leu-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ser(tBu)-OH, Fmoc-Tyr(tBu)-OH, Fmoc-Asp(OtBu)-OH, Fmoc-Asn(Trt)-OH, Fmoc-Thr(tBu)-OH, Fmoc-Phe-OH, Fmoc-Thr(tBu)-OH, Fmoc-Gly-OH, Fmoc-Gln(Trt)-OH, Fmoc-Ser(tBu)-OH and Fmoc-His(Trt)-OH of 184 μmols each, in that order. The thus-obtained carrier resin was washed with methanol and butyl ether, and then dried under reduced pressure for 3 hours. To this was added 800 μl of a mixture of TFA / $H_2O$ / thioanisole / ethanediol / ethylmethylsulfide / thiophenol (82.5 / 5 / 5 / 2.5 / 3 / 2), and left at room temperature for 6 hours, whereby the peptide was cleaved from the resin. Next, the resin was removed through filtration, and about 15 ml of ether was added to the resulting filtrate. The precipitate thus formed was taken out and lyophilized to obtain 88.9 mg of a crude product of the peptide. This crude product was purified through high-performance liquid chromatography using a reversed-phase column (column: Shiseido's CAPCELL PAK C18 SG120 S-5 μm; 30 × 250 mm). The purified product was eluted according to linear concentration gradient method with 0.1 % TFA and acetonitrile as combined with detection at 220 nm. The fractions that gave the main peaks were fractionated, and a part thereof were subjected to the above-mentioned amino acid sequencing and mass spectrometry to obtain the fraction containing OXM. This fraction was concentrated and lyophilized to obtain 25.9 mg of the entitled compound.

The synthetic peptide as obtained in the manner mentioned above was analyzed according to the reversed-phase high-performance chromatography under the 5-stage purification conditions mentioned hereinabove, along with OXM obtained in Example 1, which verified that the both was eluted at the same position.

Mass spectrometry: M + H = 4208 (theoretical: 4208)
Amino acid analysis:
Asx 3.6 (4), Glx 4.0 (4), Ser 4.0 (4), Gly 3.4 (3), His 1.0 (1), Arg 3.0 (3), Thr 4.0 (4), Ala 1.1 (1), Pro 1.0 (1) Tyr 2.0 (2), Val 1.0 (1), Met 0.9 (1), Leu 2.1 (2), Phe 2.0 (2), Lys 2.0 (2)
(Trp: not analyzed; Asx is Asp or Asn; Glx is Glu or Gln.)

INDUSTRIAL APPLICABILITY

According to the present invention, there is provided a novel peptide or pharmaceutically acceptable salts thereof,

which have a cardiotonic activity and an insulin release-promoting activity and are useful for prevention or treatment of cardiopathy or diabetes.

Sequence Listing:

Sequence Number: 1

Length of Sequence: 36

Type of Sequence: Amino acid

Number of Strand: Single-stranded

Topology: Linear

Kind of Sequence: Peptide

Origin:

Name: Eel (*Anguilla japonica*)

Tissue: Intestines

Sequence:

```
His Ser Gln Gly Thr Phe Thr Asn Asp Tyr Ser Lys Tyr
 1               5                  10

Leu Glu Thr Arg Arg Ala Gln Asp Phe Val Gln Trp Leu

     15                  20                  25

Met Asn Ser Lys Arg Ser Gly Gly Pro Thr
             30                  35
```

## Claims

1. A peptide of formula (I):
   H-His-Ser-Gln-Gly-Thr-Phe-Thr-Asn-Asp-Tyr-Ser-Lys-Tyr-Leu-Glu-Thr-Arg-Arg-Ala-Gln-Asp-Phe-Val-Gln-Trp-Leu-Met-Asn-Ser-Lys-Arg-Ser-Gly-Gly-Pro-Thr-OH (I)
   or pharmaceutically acceptable salts thereof.

2. A medicine comprising a compound of claim 1.

3. A cardiac comprising a compound of claim 1.

4. A medicine for diabetes comprising a compound of claim 1.

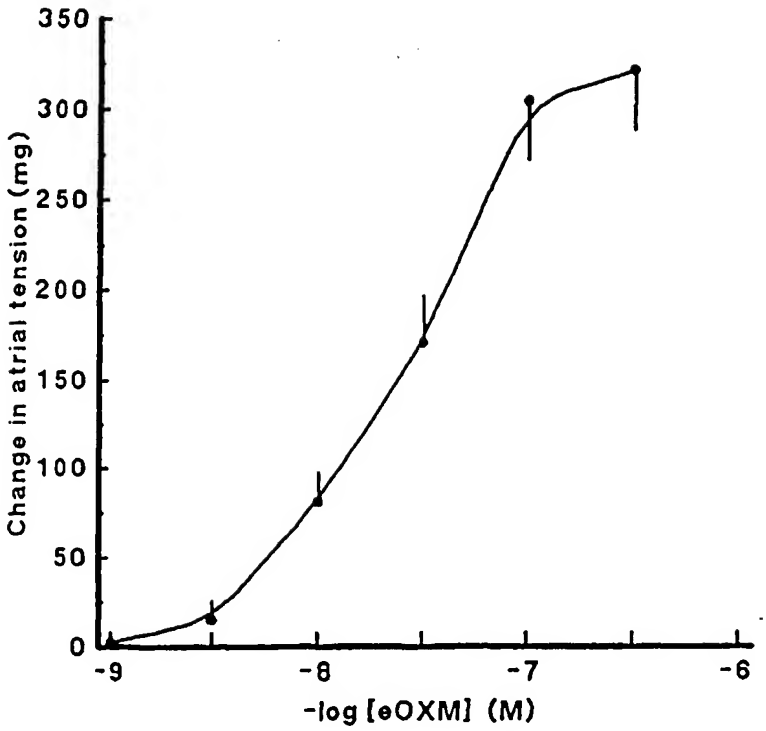



**Fig.1 Effect of Oxyntomodulin on eel atrial contoraction**

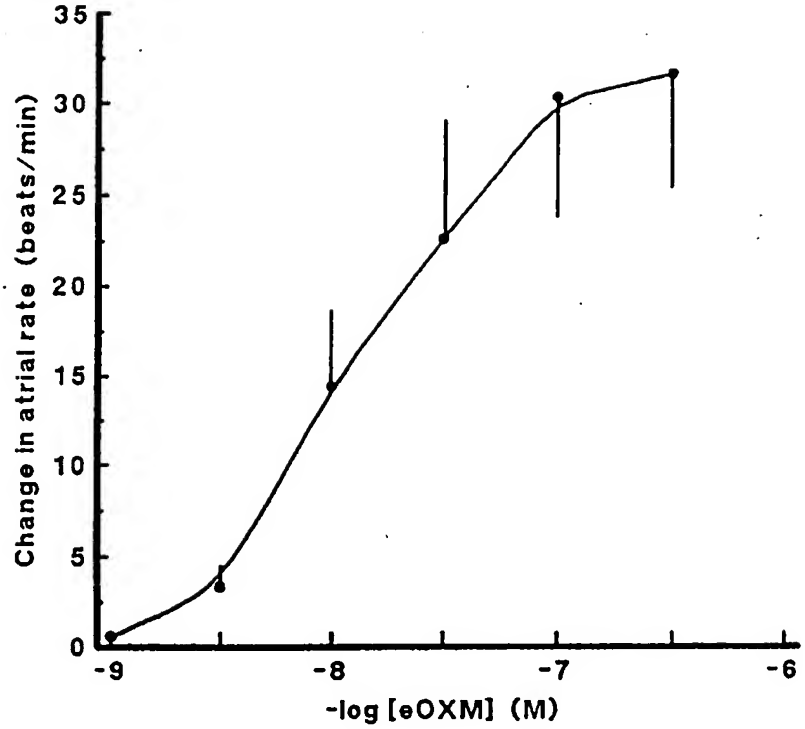
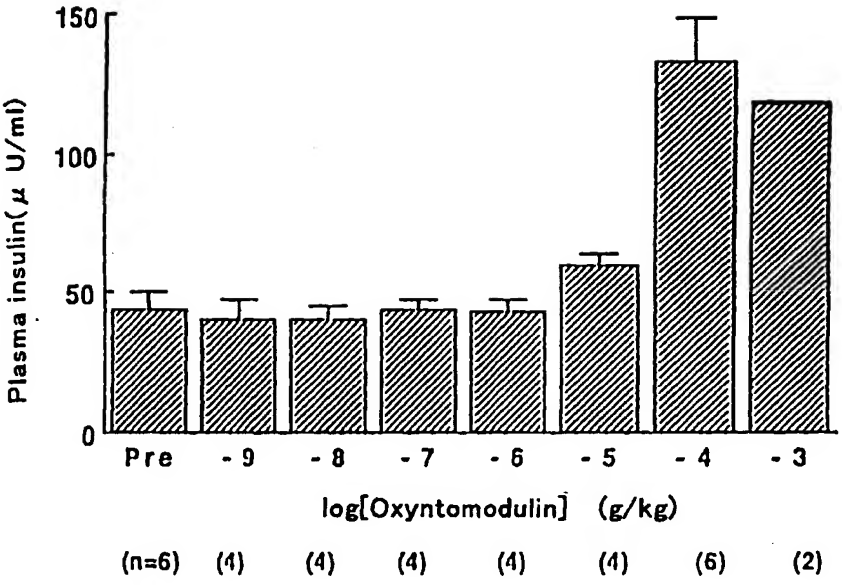



Fig.2 Effect of Oxyntomodulin on eel atrial




(Pre:Data before administration of Oxyntomodulin)

Fig.3 Effect of Oxyntomodulin on rat plasma insulin concentration

## INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP95/02269

A. CLASSIFICATION OF SUBJECT MATTER
Int. Cl$^6$ C07K14/575, A61K38/22

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
Int. Cl$^6$ C07K14/575, A61K38/22

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | GENERAL AND COMPARATIVE ENDOCRINOLOGY, Vol. 72, p. 181-189, (1988) | 1 - 4 |
| A | J. Biol. Chem., Vol. 263, No. 20, p. 9746-9751, (1988) | 1 - 4 |

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| November 30, 1995 (30. 11. 95) | December 19, 1995 (19. 12. 95) |
| Name and mailing address of the ISA/ Japanese Patent Office<br>Facsimile No. | Authorized officer<br>Telephone No. |

Form PCT/ISA/210 (second sheet) (July 1992)